Burgess et al.

Application No.: 09/186,775

Page 2

<u>IN THE CLAİMS:</u>

Please amend claims 1, 6, 13, 14, 20, and 27 as follows, without prejudice to subsequent revival of the amended subject matter.

Please cancel claims 5, 8, 9, 10, 19, 22, 23, and 24, without prejudice to subsequent revival.

Please add new claims 28-37.

All pending claims are provided for the Examiner's convenience.

1. (once amended) A plant containing a plant cell comprising a first and a second expression cassette located at the same locus on each of two homologous chromosomes, wherein:

the first expression cassette present on a first chromosome homolog comprises a first plant promoter operably linked to a first polynucleotide sequence encoding a first polypeptide, wherein a recombinase site is present between the first promoter and the first polynucleotide sequence;

the second expression cassette present on a second chromosome homolog comprises the first plant promoter inoperably linked to the first polynucleotide sequence, wherein an intervening expression cassette is flanked by two recombinase sites and situated between the first promoter and the first polynucleotide sequence of the second expression cassette, the intervening expression cassette comprising a second plant promoter operably linked to a second polynucleotide sequence encoding a second polypeptide;

wherein at least the first or the second plant promoter is a non-constitutive promoter; wherein at least the first or the second polynucleotide encodes an amino acid sequence from a miclease; and

wherein the presence of the first and second polypeptides in a cell is lethal to the cell.

2. (as filed) The plant of claim 1, wherein the recombinase sites are lox sites.



Burgess *et al.*Application No.: 09/186,775
Page 3

3. (as filed) The plant of claim 1, wherein the first polypeptide is a transactivator protein.

- 4. (as filed) The plant of claim 1, wherein the intervening expression cassette is in reverse orientation with respect to the second expression cassette.
- 6. (once amended) The plant of claim [5] 1, wherein at least the first or the second [polypeptide] polynucleotide encodes an amino acid sequence from [is] a ribonuclease.
 - 7. (as filed) The plant of claim 6, wherein the ribonuclease is Barnase.
- 11. (as filed) The plant of claim 1, wherein the first or the second promoter is a tissue-specific promoter.
- 12. (as filed) The plant of claim 1, wherein the first and second promoters are each functional in tapetal cells.

13. (once amended) The plant of claim 1, wherein the first and second polypeptides each comprise a separate subsequence of a single functional <u>nuclease</u> polypeptide.

Sul

14. (once amended) A method of modifying cellular function in a plant, the method comprising the steps of:

introducing into a plant a first expression cassette comprising a first plant promoter operably linked to a first polynucleotide encoding a first polypeptide, wherein a recombinase site is present between the first promoter and the first polynucleotide;

introducing into the plant a second expression cassette comprising the first plant promoter inoperably linked to a polynucleotide encoding the first polypeptide, wherein an intervening expression cassette is flanked by recombinase sites and situated between the first promoter and the first polypeptide of the second expression cassette, the intervening expression

23

Burgess et al.

Application No.: 09/186,775

Page 4

cassette comprising a plant promoter operably linked to a polymeteleotide encoding a second polypeptide;

wherein at least the first or the second plant promoter is a non-constitutive promoter; wherein at least the first or the second polynucleotide encodes an amino acid sequence from a nuclease; and

wherein the presence of the first and second polypeptides in a cell is lethal to the cell.

- 15. (as filed) The method of claim 14, wherein the two expression cassettes are introduced through a sexual cross and the two expression cassettes are present on chromosome homologs.
- 16. (as filed) The method of claim 14, wherein the recombinase sites are lox sites.
- 17. (as filed) The method of claim 14, wherein the first polypeptide is a transactivator protein.
- 18. (as filed) The method claim 14, wherein the intervening expression cassette is in reverse orientation with respect to the second expression cassette.

20. (once amended) The method of claim [19] 14, wherein at least the first or the second [polypeptide] polynucleotide encodes an amino acid sequence from [is] a ribonuclease.

- 21. (as filed) The method of claim 20, wherein the ribonuclease is Barnase.
- 25. (as filed) The method of claim 14, wherein the first or the second promoter is a tissue-specific promoter.

B

B4

Burgess *et al.*Application No.: 09/186,775
Page 5

26. (as filed) The method of claim 14, wherein the first and second promoters are each functional in tapetal cells.

B3.

2) (once amended) The method of claim 14, wherein the first and second polypeptides each comprise a separate subsequence of a single functional <u>nuclease</u> polypeptide.

- 28. (new) The plant of claim 1, wherein both the first and the second promoters are non-constitutive promoters.
- 29. (new) The plant of claim 1, wherein the first and second promoters have overlapping specificities.
- 30. (new) The plant of claim 1, wherein the first or the second promoter is a seed coat-specific promoter.
- 31. (new) The plant of claim 6, wherein the ribonuclease is ribonuclease T1 or binase.
- 32. (new) The plant of claim 6, wherein the first and second polypeptides each comprise a separate subsequence of a single functional ribonuclease polypeptide.
- 33. (new) The plant of claim 14, wherein both the first and the second promoters are non-constitutive promotes.
- 34. (new) The method of claim 14, wherein the first and second promoters have overlapping specificities.
- 35. (new) The method of claim 14, wherein the first or the second promoter is a seed coat-specific promoter.

B6